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sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Anti-Activin A
produced in goat, affinity isolated antibody

Catalog Number **A1594**

Product Description

Anti-Activin A is produced in goat using as immunogen a purified recombinant human Activin A, expressed in CHO cells. The antibody is purified using human Activin A affinity chromatography.

Anti-Activin A neutralizes human activin A and recognizes recombinant human activin A by direct ELISA, immunoblotting and immunohistochemistry.

Activin-A has been recognized for its range of activities involving growth and differentiation of several tissues from different species [1-3] It plays a key role in production and regulation of hormones such as FSH, LH, GnRH, and ACTH. Activin also influences erythropoiesis and the potentiation of erythroid colony formation, oxytocin secretion, paracrine, and autocrine regulation.[4]

Activin-A (β_A-β_A) is a disulfide-linked dimeric protein secreted by Sertoli cells in the testis and grandulosa cell in the ovary. In the early studies, this peptide was thought to be an inhibin and not recognized as a unique compound.[4,5] Activins and inhibins have been further characterized and include 3 separate peptides exhibiting a combination of +α, β_A, and β_B subunits. The C, D, and E-β subunits have also been cloned.[4] Activins are homodimers or heterodimers made up of the β subunit isoforms. Mammalian activin-A is identified as the β_A-β_A form. Bovine, porcine, human, and murine activin-A demonstrates 98% homology. These compounds are classified as members of the TGF-β super family due to amino acid homology with respect to the conservation of 7 of the 9 Cysteine residues common to all TGF-β forms.[4]

Reagent

Lyophilized from 0.2 μm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Prior to reconstitution, store at −20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at −20 °C. Avoid repeated freezing and thawing

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2 μm filtered phosphate buffered saline to produce a 0.1 mg/mL stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Procedure

Neutralization of Bioactivity

Human Activin A induces hemoglobin expression in K562 cells in a dose dependent manner. To measure the ability of the antibody to neutralize the bioactivity, recombinant human activin A is incubated with various concentrations of the antibody for 1 hour at 37 °C in a 96 well plate. Following this preincubation period, K562 cells were added. The assay mixture in a total volume of 200 μL per well, containing antibody at the concentrations indicated (0.01 μg/mL-100 μg/mL), recombinant human activin A at 7.5 ng/ml, and cells at 2.5×10^4 cells/ml are incubated at 37 °C for 4 days in a humidified CO_2 incubator. At the end of the incubation, the hemoglobin level in cell lysate is measured for its pseudoperoxidase activity. The ND_{50} is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.

Product Profile

Neutralization: a working antibody concentration of 2-6 μg/ml will neutralize 50% of the bioactivity due to 7.5 ng/ml recombinant human activin A using K562 cells.

Immunoblotting: a working antibody concentration of 0.1 μg/mL was determined

Immunohistochemistry: a recommended antibody concentration of 5-15 μg/mL is determined using immersion fixed paraffin-embedded human breast cancer tissue.

Due to autofluorescence of tissues dissected from non-human primates, the use of fluorescent probes, such as FITC or Cy3™, is not recommended unless autofluorescence is quenched.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

Endotoxin level is <10 ng per mg antibody as determined by the LAL method.

References

1. Smith, J., et al., Expression of a *Xenopus* homolog of Brachyury (T) is an immediate-early response to mesoderm induction. *Cell*, **67**, 79 (1991).
2. de Winter, J., et al., Activin is produced by rat Sertoli cells *in vitro* and can act as an autocrine regulator of Sertoli cell function. *Endocrinology*, **132**, 975 (1993).
3. Sporn, M.B., and Roberts, A.B., eds., Peptide Growth Factors and Their Receptors, Springer-Verlag Heidelberg, Vol I, p429 (1991).
4. Sporn, M.B., and Roberts, A.B., eds. Peptide Growth factors and Their Receptors, Springer-Verlag Heidelberg, Vol II, pp 217-235 (1991).
5. De Jong, F., et al., Effects of factors from ovarian follicular fluid and Sertoli cell culture medium on *in vivo* and *in vitro* release of pituitary gonadotrophins in the rat: an evaluation of systems for the assay of inhibin. *J. Reprod. Fertil*., **26**, 47 (1979).

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